Bare Minimal Microscopy Reporting Requirements Checklist

A detailed and accurate description of microscopy methods, including hardware specifications and other metadata, is essential for ensuring reproducibility and transparency in imaging experiments. To address this need, WG11 of QUAREP-LiMi has developed a universal minimal checklist for reporting microscopy data. This checklist outlines the bare minimum requirements applicable to most microscopy applications, aiming to enhance reproducibility in image-based science.

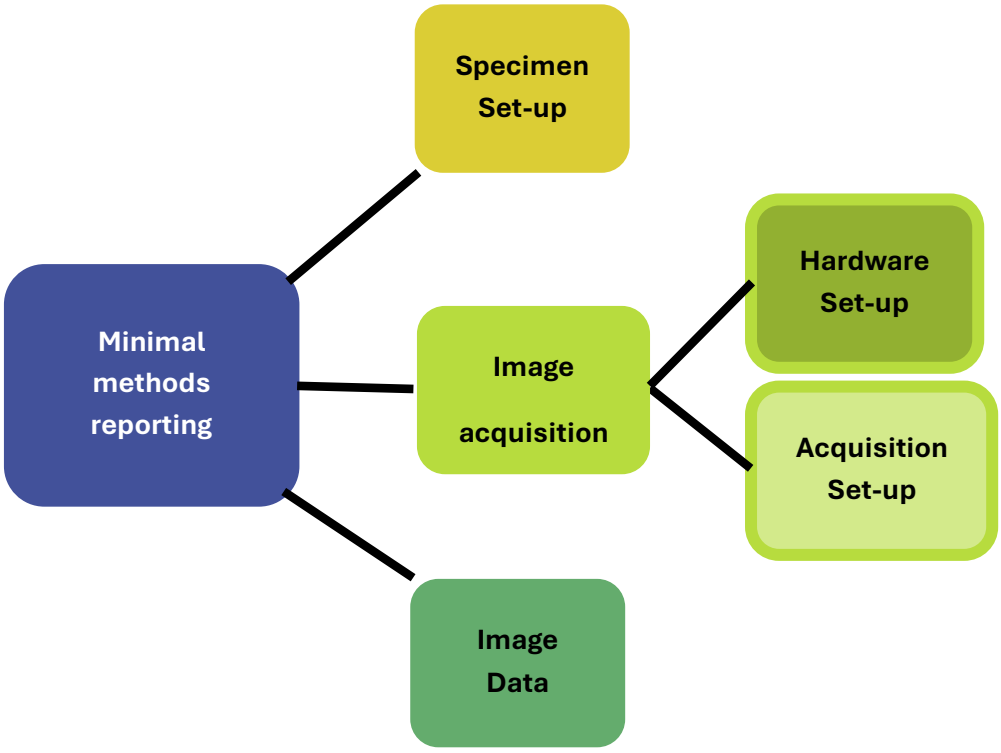

Figure 1. Categories used in the checklist for reporting fluorescence microscopy methods, structured according to the Recommended Metadata for Biological Images (REMBI)[1] framework and the NBO-Q[2] Metadata Model

Categories		Examples
Specimen set-up		
Sample mounting	Cover glass (cover glass number or thickness; coating)	Samples were grown on #1.5H cover glass (Marienfeld), coated with 1 mg/ml collagen type I (Sigma, C8919).
	Mounting medium or imaging medium (name and manufacturer)	Prior to imaging, samples were mounted in Slowfade Glass mounting medium (ThermoFisher).
Sample labelling*	Fluorescent protein (specific variant or probe)	mGFPmut3, GCaMP6f
	Dye (name, manufacturer and concentration)	MitoTracker Green at 1 µg/ml final Secondary antibody conjugated to AlexaFluor 647
Hardware set-up		
Microscope stand	Description (manufacturer; model; inverted or upright)	Microscopy imaging experiments were performed on a Nikon Ti2 inverted microscope stand.
Modalities and modules/add-on	Specify the modalities and modules used**	Microscope stand was equipped with a Yokogawa spinning disk CSU-W1 and a SORA module.
		For phase contrast imaging, we used a phase contrast objective and a respective phase plate in the condenser.
		Fluorescent images were captured on a Zeiss Observer.Z1 widefield microscope equipped with an Apotome module.
Objective and additional magnification	Full designation (description, specification) found on the barrel of the objective (magnification, numerical aperture, correction type, and immersion type)	Images were acquired using a 100x/1.45 DIC Plan Apochromat, oil immersion objective.
	Additional Magnification (magnification changer)	In addition, a 1.5x optovar was inserted in the lightpath.
Light source	Manufacturer and model (for non-laser light sources)	Samples were illuminated using an LED light engine (Spectra X, Lumencor).
	Type (for non-laser light sources) (e.g., LED, mixed metal halide, mercury)	
	Specify the excitation wavelength used (for laser-based)	DAPI excitation was performed using a 405 nm diode laser.
Wavelength selection	Specific filters or filter cubes (excitation filter center wavelength/FWHM, emission filter center wavelength/FWHM, and optionally company, filter name)	AlexaFluor 488 was imaged using filter cube 38 HE (Zeiss, BP 470/40 Ex, DC495 dichroic, BP525/50Em)
	Adjustable wavelength selector (e.g., spectral detection in a point scanner), cut on/cutoff wavelengths	eGFP emission was detected with a spectral detector between 500 nm and 544 nm, with a spectral width of 8.9 nm for the single detection elements.
Detection system (as applicable)	Camera (manufacturer and model)	An Orca Flash 4.0 (Hamamatsu) monochrome camera
	Point detector (type)	A 32-channel GaAsP detector was used to detect the emitted signal.
Acquisition set-up	All acquisition settings optimised to acquire the image must be reported, and should include but not be limited to:	
Acquisition settings	Camera based: exposure time	The DAPI channel was captured with 30 ms exposure time (camera-based)
	Point detector based: pinhole size (in AU), pixel dwell or scan speed, simultaneous or sequential acquisition.	The images were captured sequentially, with a pixel dwell time of 2 µs and a 1 AU pinhole and no averaging
	Final effective image pixel size (in the image)	Final image pixel size was 0.065 um/pixel.
	Z-stack settings (z-step increment, number of steps, total range)	Images were acquired as 15 µm range z-stacks with a 100 nm z-step interval.
	Time series settings (time increment and total acquisition time)	Time-lapse imaging was performed for 2.5 hours with a 10 min interval.
	Tiling settings	Tiling was performed with a 10% overlap.
Acquisition software	Name, manufacturer, and version	NIS-Elements AR V5.21 (Nikon) Micro-manager 2.0.0.

Table 1. Minimal Requirements for reporting microscopy methods

Categories		Examples	Machine readable NBO-Q* alignment
Specimen set-up			
Sample mounting	Cover glass (cover glass number or thickness; coating)	Samples were grown on #1.5H cover glass (Marienfeld), coated with 1 mg/ml collagen type I (Sigma, C8919).	CoverGlass/CoverGlassNo CoverGlass/Thickness CoverGlass/Coating
	Mounting medium or imaging medium (name and manufacturer)	Prior to imaging, samples were mounted in Slowfade Glass mounting medium (ThermoFisher).	MountingMedium/Model MountingMedium/Manufacturer
Sample labelling*	Fluorescent protein (specific variant or probe)	mGFPmut3, GCaMP6f	
	Dye (name, manufacturer and concentration)	MitoTracker Green at 1 µg/ml final Secondary antibody conjugated to AlexaFluor 647	
Hardware set-up			
Microscope stand	Description (manufacturer; model; inverted or upright)	Microscopy imaging experiments were performed on a Nikon Ti2 inverted microscope stand.	MicroscopeStand/Manufacturer MicroscopeStand/Model MicroscopeStand subtype (Inverted, Upright)
Modalities and modules/add-on	Specify the modalities and modules used**	Microscope stand was equipped with a Yokogawa spinning disk CSU-W1 and a SORA module.	Pixels/Channel/IlluminationType (Wide-field_Fluorescence, Confocal_Fluorescence_array-raster-scan, Confocal_Fluorescence_spinning disk)
		For phase contrast imaging, we used a phase contrast objective and a respective phase plate in the condenser.	
		Fluorescent images were captured on a Zeiss Observer.Z1 widefield microscope equipped with an Apotome module.	
Objective and additional magnification	Full designation (description, specification) found on the barrel of the objective (magnification, numerical aperture, correction type, and immersion type)	Images were acquired using a 100x/1.45 DIC Plan Apochromat, oil immersion objective.	Objective/Magnification Objective/LensNA Objective/Correction Objective/ImmersionType
	Additional Magnification (magnification changer)	In addition, a 1.5x optovar was inserted in the lightpath.	MagnificationChanger
Light source	Manufacturer and model (for non-laser light sources) Type (for non-laser light sources) (e.g., LED, mixed metal halide, mercury)	Samples were illuminated using an LED light engine (Spectra X, Lumencor).	LightSource subtype (Arc, Filament, GenericLightSource, Laser, LightEmittingDiode, MultiLaserEngine) LightSource/Manufacturer
	Specify the excitation wavelength used (for laser-based)	DAPI excitation was performed using a 405 nm diode laser.	LightSource/Model LightSource/Laser LightSource/PeakWavelength
Wavelength selection	Specific filters or filter cubes (excitation filter center wavelength/FWHM, emission filter center wavelength/FWHM, and optionally company, filter name)	AlexaFluor 488 was imaged using filter cube 38 HE (Zeiss, BP 470/40 Ex, DC495 dichroic, BP525/50Em)	FilterCube Filter/TransmittanceRange/Wavelength Filter/TransmittanceRange/FWHMBandwidth Filter/Manufacturer Filter/Model
	Adjustable wavelength selector (e.g., spectral detection in a point scanner), cut on/cutoff wavelengths	eGFP emission was detected with a spectral detector between 500 nm and 544 nm, with a spectral width of 8.9 nm for the single detection elements.	Dichroic/TransmittanceRange/Wavelength
Detection system (as applicable)	Camera (manufacturer and model)	An Orca Flash 4.0 (Hamamatsu) monochrome camera	Camera/Manufacturer Camera/Model
	Point detector (type)	A 32-channel GaAsP detector was used to detect the emitted signal.	PointDetector subtype (Multialkali/GaAsP)PhotomultiplierTube, PhotoDiode, HybridPhotoDetector)
Acquisition set-up	All acquisition settings optimised to acquire the image must be reported, and should include but not be limited to:		
Acquisition settings	Camera based: exposure time	The DAPI channel was captured with 30 ms exposure time (camera-based)	Pixels/Plane/ExposureTime
	Point detector based: pinhole size (in AU), pixel dwell or scan speed, simultaneous or sequential acquisition.	The images were captured sequentially, with a pixel dwell time of 2 µs and a 1 AU pinhole and no averaging	PinholeSettings/Aperture ConfocalScannerSettings/MultiChannelMode/Parallel or Sequential ConfocalScannerSettings/ScanningFrequency
	Final effective image pixel size (in the image)	Final image pixel size was 0.065 um/pixel.	Pixels/PhysicalSizeX Pixels/PhysicalSizeY
	Z-stack settings (z-step increment, number of steps, total range)	Images were acquired as 15 µm range z-stacks with a 100 nm z-step interval.	Pixels/PhysicalSizeZ Pixels/SizeZ
	Time series settings (time increment and total acquisition time)	Time-lapse imaging was performed for 2.5 hours with a 10 min interval.	Pixels/Plane/TimeIncrement
	Tiling settings	Tiling was performed with a 10% overlap.	Pixels/Plane/PixelDwellTime
Acquisition software	Name, manufacturer, and version	NIS-Elements AR V5.21 (Nikon) Micro-manager 2.0.0.	AcquisitionSoftware/Name AcquisitionSoftware/Developer AcquisitionSoftware/Version

Table 2. Minimal requirements for reporting microscopy methods, including machine-readable metadata (NBO-Q Metadata Model; grey column)

*__Sample preparation__ is critical for a rigorous and reproducible experiment, but it belongs to other sections within the material and methods. A detailed protocol describing growth conditions, transfection conditions, imaging environmental conditions if applicable (live cell imaging), labeling method (fixation conditions, permeabilization, antibody concentration...) should be included. We are including the fluorescent protein variant or the used fluorescent dye because this is a specification that it is usually often missing in current methods section but essential for judging the hardware set-up and the acquisition settings.

**__Modality__ is a microscopy technique such as fluorescence widefield, phase contrast, fluorescence, confocal or STED, 2photon, light-sheet, SIM, etc...

Modules are hardware devices or add-ons enabling a modality such as a TIRF arm, confocal point scanner, spinning disk

#NBO-Q[2,3] alignment provided as Model Element/Attribute. Alignment with the 4DN-BINA-OME-QUAREP (NBO-Q) data model provides the necessary information for the development and use of various automated processes. The NBO-Q data model is living tool created by and for the community. Its refinement reflects interactions within the imaging community. As of year 2024, QUAREP-LiMi is the curator of the data model and has implemented a process for future revision.

Acknowledgments
We strongly encourage **acknowledgment of core facilities** and instrumentation grant(s). Be aware that it is often a legal requirement to acknowledge that your research is done using government-funded instruments. Ensure you include the source of funds for the core facility.

Example Number 1- Widefield:
Images were acquired with an IX83P2ZF inverted wide-field microscope stand (Olympus) equipped with the following motorized components: a linear-encoded Olympus IX3-SSU stage with ultrasonic motor and a Z-deck motor with 0.02 µm resolution. For epifluorescent images, a SOLA SE Light Engine (Lumencor) was used in combination with Chroma filter set #49002 (Chroma, ET470/40X, T495lprx, ET525/50m), filter set #49031 (Chroma, ET569/25x, T588lpxr, ET615/40m) and filter set #49006 (Chroma, ET620/60X, T660lprx, ET700/75m). Emission was collected using a Fusion-BT sCMOS camera (Hamamatsu) using a 60x/1.4 NA PlanApochromat oil immersion objective without additional magnification lenses. The final image pixel size was 0.095 µm/pixel. Z-stack images were collected at 0.3 µm steps over a range of 30 µm with the shutter closed during Z-movement. Acquisition was controlled with CellSens software, v4.2.1 (Olympus).

Example Number 2- Spinning disk confocal:
Samples were imaged with a Yokogawa CSU-W1 spinning disk (50 µm pinhole size, Nikon) confocal unit attached to a fully motorized Ti inverted microscope stand equipped with a linear-encoded motorized stage (Nikon) with a PI 250 µm range Z piezo insert. Images were captured using an Andor Zyla 4.2 plus sCMOS camera through a 100x/1.45 NA Plan Apo DIC oil immersion objective with Cargille Type 37 immersion oil. The final pixel size of the image was 0.065 µm/pixel. Green and red fluorescence were collected by illuminating the sample with a solid-state 488 nm diode laser line and a solid state 561 nm DPSS laser respectively from a Toptica iChrome MLE laser launch. A Di01T405/488/568/647 multi-bandpass dichroic mirror (Semrock) was used for both channels, and the final emission was acquired sequentially with ET525/36 nm and ET605/52 nm emission filters (Chroma) using a filter wheel placed within the scan unit for green and red channels respectively. Volume images were acquired as 15 µm range z-stacks with a 100 nm z-step interval using either the PI piezo z-device or the Ti z-drive, capturing both channels at each z-plane (data capture order xycz). The NIS Elements AR 5.02 acquisition software (Nikon) was used to acquire the data which were saved in the ND2 file format.

References:

1. Colors of this figure follow the scheme of the REMBI paper from:
   Sarkans, U., Chiu, W., Collinson, L. et al. REMBI: Recommended Metadata for Biological Images—enabling reuse of microscopy data in biology. Nat Methods 18, 1418–1422 (2021). https://doi.org/10.1038/s41592-021-01166-8

2. NBO-Q repo:
   https://github.com/WU-BIMAC/NBOMicroscopyMetadataSpecs/tree/master/Model/in%20progress/v02-10

3. Hammer, Mathias, Maximiliaan Huisman, Alessandro Rigano, Ulrike Boehm, James J. Chambers, Nathalie Gaudreault, Alison J. North, et al. Towards Community-Driven Metadata Standards for Light Microscopy: Tiered Specifications Extending the OME Model. *Nature Methods*, December 3, 2021, 1–14. https://doi.org/10.1038/s41592-021-01327-9

4. Schmied, C., Nelson, M.S., Avilov, S. et al. Community-developed checklists for publishing images and image analyses. Nat Methods 21, 170–181 (2024). https://doi.org/10.1038/s41592-023-01987-9